

WHAT IS CLAIMED IS:

1. A method of creating a nucleic acid product terminated at a selected base, comprising
creating a substantially double stranded nucleic acid template comprising at least a first break on
5 at least one strand, and contacting said template with an effective polymerase and a terminating
composition comprising at least a first terminating nucleotide, wherein the base of said
terminating nucleotide corresponding to said selected base, under conditions effective to produce
a nucleic acid product terminated at a selected base.

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2. The method of claim 1, comprising creating a substantially double-stranded nucleic acid
template comprising at least a first break on only one strand.

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3. The method of claim 1, wherein said template is created by contacting a substantially
double-stranded nucleic acid with a combined effective amount of at least a first and second
breaking enzyme combination.

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4. The method of claim 3, wherein said template is created by generating a substantially
double-stranded nucleic acid comprising at least a first uracil residue, and contacting said nucleic
acid with a combined effective amount of a first, uracil DNA glycosylase enzyme and a second,
endonuclease IV enzyme or endonuclease V enzyme.

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5. The method of claim 4, wherein said at least a first enzyme is uracil DNA glycosylase
and said at least a second enzyme is endonuclease V.

6. The method of claim 1, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a chemical cleavage composition.
- 5 7. The method of claim 1, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of at least a first nuclease enzyme.
- 10 8. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first specific break on at least one strand.
- 15 9. The method of claim 8, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of at least a first specific nuclease enzyme.
- 20 10. The method of claim 9, wherein said specific nuclease enzyme is f1 endonuclease, fd endonuclease or a restriction endonuclease.
11. The method of claim 10, wherein said specific nuclease enzyme is f1 endonuclease.
- 25 12. The method of claim 8, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a specific chemical cleavage composition.
- 30 13. The method of claim 12, wherein said specific chemical cleavage composition comprises a triple helix forming composition.

14. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first random break on at least one strand.

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15. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first random break on only one strand.

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16. The method of claim 15, wherein said template is created by generating a substantially double-stranded nucleic acid comprising at least a first randomly positioned exonuclease-resistant nucleotide, and contacting said nucleic acid with an effective amount of an exonuclease.

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17. The method of claim 16, wherein said exonuclease-resistant nucleotide is a deoxyribonucleotide phosphorothioate or a deoxyribonucleotide boranophosphate.

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18. The method of claim 16, wherein said exonuclease is exonuclease III.

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19. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of at least a first randomly-breaking nuclease enzyme.

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20. The method of claim 19, wherein said randomly-breaking nuclease enzyme is deoxyribonuclease I.

21. The method of claim 19, wherein said randomly-breaking nuclease enzyme is *CviJI* restriction endonuclease.

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22. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with a combined effective amount of at least a first and second randomly-breaking enzyme combination.

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23. The method of claim 22, wherein the first and second randomly-breaking enzymes are distinct, frequent-cutting restriction endonucleases.

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24. The method of claim 23, wherein said distinct, frequent-cutting restriction endonucleases are selected from the group consisting of *Tsp509I*, *MaeII*, *TaiI*, *AhuI*, *CviJI*, *NlaIII*, *MspI*, *HpaII*, *BstUI*, *BfaI*, *DpnII*, *MboI*, *Sau3AI*, *DpnI*, *Chal*, *HinPI*, *HhaI*, *HaeIII*, *Csp6I*, *RsaI*, *TaqI* and *MseI*.

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25. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a randomly-breaking chemical cleavage composition.

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26. The method of claim 25, wherein said randomly-breaking chemical cleavage composition comprises or reacts to produce a hydroxyl radical.

27. The method of claim 26, wherein said randomly-breaking chemical cleavage composition comprises a chelating agent, a metal ion, a reducing agent and a peroxide.

5 28. The method of claim 27, wherein said randomly-breaking chemical cleavage composition comprises EDTA, an Fe²⁺ ion, sodium ascorbate and hydrogen peroxide.

10 29. The method of claim 26, wherein said randomly-breaking chemical cleavage composition comprises a compound that produces a hydroxyl radical upon contact with defined wavelengths of light.

15 30. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of gamma irradiation.

20 31. The method of claim 15, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective mechanical breaking process.

32. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of a hydrodynamic force.

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33. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of sonication.

34. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of nebulization.

5 35. The method of claim 15, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of freezing and thawing.

36. The method of claim 1, wherein said break is a nick comprising a 3' hydroxyl group.

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37. The method of claim 36, wherein said effective polymerase has 5' to 3' exonuclease activity.

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38. The method of claim 36, wherein said effective polymerase has strand displacement activity.

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39. The method of claim 36, wherein said effective polymerase is *E. coli* DNA polymerase I, *Taq* DNA polymerase, *S. pneumoniae* DNA polymerase I, *Tfl* DNA polymerase, *D. radiodurans* DNA polymerase I, *Tth* DNA polymerase, *Tth* XL DNA polymerase, *M. tuberculosis* DNA polymerase I, *M. thermoautotrophicum* DNA polymerase I, Herpes simplex-1 DNA polymerase, *E. coli* DNA polymerase I Klenow fragment, vent DNA polymerase, thermosequenase or a wild-type or modified T7 DNA polymerase.

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40. The method of claim 39, wherein said effective polymerase is *E. coli* DNA polymerase I, *M. tuberculosis* DNA polymerase I or *Taq* DNA polymerase.

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41. The method of claim 1, wherein said break is a gap comprising a 3' hydroxyl group.
- 5 42. The method of claim 41, wherein said effective polymerase is *E. coli* DNA polymerase I, *Taq* DNA polymerase, *S. pneumoniae* DNA polymerase I, *Tfl* DNA polymerase, *D. radiodurans* DNA polymerase I, *Tth* DNA polymerase, *Tth* XL DNA polymerase, *M. tuberculosis* DNA polymerase I, *M. thermoautotrophicum* DNA polymerase I, Herpes simplex-1 DNA polymerase, *E. coli* DNA polymerase I Klenow fragment, T4 DNA polymerase, vent DNA polymerase, thermosequenase or a wild-type or modified T7 DNA polymerase.
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- 15 43. The method of claim 42, wherein said effective polymerase is *E. coli* DNA polymerase I, *M. tuberculosis* DNA polymerase I, *Taq* DNA polymerase or T4 DNA polymerase.
- 20 44. The method of claim 1, wherein said terminating composition comprises a terminating dideoxynucleotide triphosphate, the base of which corresponds to said selected base.
- 25 45. The method of claim 1, wherein said terminating composition comprises a terminating deoxynucleotide triphosphate, the base of which corresponds to said selected base.
- 30 46. The method of claim 1, wherein said terminating nucleotide comprises a detectable label or an isolation tag that is incorporated into said nucleic acid product.
47. The method of claim 1, wherein said template comprises a detectable label or an isolation tag.

48. The method of claim 1, wherein said template and said terminating nucleotide each comprise a detectable label or an isolation tag.

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49. The method of claim 1, wherein said template or said terminating nucleotide comprise a radioactive, enzymatic or fluorescent label.

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50. The method of claim 1, wherein said template or said terminating nucleotide comprise a biotin molecule isolation tag.

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51. The method of claim 1, further comprising detecting said nucleic acid product.

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52. The method of claim 51, further defined as a method for sequencing a nucleic acid, the method comprising detecting said nucleic acid product under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid.

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53. The method of claim 51, further defined as a method for mapping a nucleic acid, the method comprising detecting said nucleic acid product under conditions effective to determine the position of said nucleic acid relative to said nucleic acid product.

54. The method of claim 53, comprising:

- a) creating a population of substantially double-stranded nucleic acid templates comprising at least a first random break on at least one strand;
- b) contacting said templates with an effective polymerase and at least a first degradable extension-producing composition comprising three non-degradable extending nucleotides and one degradable nucleotide, under conditions and for a time effective to produce a population of degradable nucleic acid products comprising said degradable nucleotide;
- c) removing said degradable extension-producing composition from contact with said templates;
- d) contacting said population of degradable nucleic acid products with an effective polymerase and at least a first nondegradable extending and terminating composition comprising four non-degradable extending deoxynucleotides, at least one of said non-degradable extending deoxynucleotides comprising a detectable label or an isolation tag, under conditions and for a time effective to produce a population of terminated nucleic acid products comprising a degradable region and a nondegradable region;
- e) contacting said population of terminated nucleic acid products with an effective amount of a degrading composition to degrade said degradable region, thereby producing nested nucleic acid products; and
- f) detecting said nested nucleic acid products under conditions effective to determine the position of said nucleic acid relative to said nucleic acid product.

55. The method of claim 54, wherein said degradable nucleotide comprises a uracil base, and wherein said degrading composition comprises a combined effective amount of a uracil DNA glycosylase enzyme and an endonuclease IV or an endonuclease V enzyme.

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56. The method of claim 51, wherein said nucleic acid product comprises a detectable label, and said nucleic acid product is detected by detecting said label.

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57. The method of claim 51, wherein said nucleic acid product comprises an isolation tag, and said nucleic acid product is purified using said isolation tag prior to detection.

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58. The method of claim 51, wherein said nucleic acid product is separated prior to detection.

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59. The method of claim 58, wherein said nucleic acid product is separated by electrophoresis, mass spectroscopy, FPLC or HPLC prior to detection.

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60. The method of claim 15, wherein at least a first specified base is incorporated at said random break of said template prior to producing the nucleic acid product terminated at the selected base.

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61. The method of claim 15, comprising contacting said template with an effective polymerase and extending and terminating composition under conditions effective to produce a nucleic acid product comprising at least one specified base prior to termination at said selected base.

62. The method of claim 60, comprising contacting said template with four extending nucleotides and said terminating nucleotide under conditions effective to produce a population of nucleic acid products terminated at said selected base.

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63. The method of claim 62, wherein at least one of said extending nucleotides is a degradable nucleotide.

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64. The method of claim 60, further defined as a method for identifying a selected dinucleotide sequence in said nucleic acid template, the dinucleotide sequence being the complement of said specified and selected base, the method comprising:

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a) blocking said template by contacting with a blocking composition comprising the three dideoxynucleotide triphosphates that do not contain the specified base;

b) removing said blocking composition from contact with said template;

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c) contacting said template with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing said specified base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing said selected base, under conditions effective to produce a nucleic acid product terminating with a dinucleotide sequence of said specified and selected base; and

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d) detecting said nucleic acid product under conditions effective to identify the selected dinucleotide sequence in said nucleic acid template.

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65. The method of claim 15, further defined as a method for identifying a selected dinucleotide sequence of a first and second base in a nucleic acid template, said method comprising:

- 5 a) blocking said template by contacting with a blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;
- 10 b) removing said blocking composition from contact with said template;
- 15 c) contacting said template with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said second base, under conditions effective to produce a nucleic acid product terminating with a dinucleotide sequence complementary to said first and second base; and
- 20 d) detecting said nucleic acid product under conditions effective to identify said selected dinucleotide sequence in said nucleic acid template.

66. The method of claim 65, wherein step (c) comprises contacting said template with a single extending and terminating composition that comprises both said extending deoxynucleotide triphosphate and said terminating dideoxynucleotide triphosphate.

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67. The method of claim 65, wherein step (c) comprises first contacting said template with an extending composition that comprises said extending deoxynucleotide triphosphate, and then contacting said template with a distinct terminating composition that comprises said terminating dideoxynucleotide triphosphate.

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68. The method of claim 67, wherein step (c) comprises, in sequence, contacting said template with an extending composition that comprises said extending deoxynucleotide triphosphate, removing said extending composition from contact with said template, and contacting said template with a distinct terminating composition that comprises said terminating dideoxynucleotide triphosphate.
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- 10 69. The method of claim 15, wherein at least a first and a second specified base are incorporated at said random break of said template prior to producing said nucleic acid product.
- 15 70. The method of claim 15, comprising subjecting said template to a series of blocking and extending reactions prior to contact with said terminating composition, thereby producing an extended nucleic acid product comprising a series of additional bases preceding the selected, terminating base.
- 20 71. The method of claim 69, further defined as a method for identifying a selected trinucleotide sequence in said nucleic acid template, the trinucleotide sequence being the complement of said first and second specified bases and said selected base, the method comprising:
- 25 a) blocking said template by contacting with a first blocking composition comprising three dideoxynucleotide triphosphates that do not contain the first specified base;
- b) removing said first blocking composition from contact with said template;

- c) extending said template by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing said first specified base;
- 5 d) removing said first extending composition from contact with said template;
- e) blocking said template by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the second specified base;
- 10 f) removing said second blocking composition from contact with said template;
- g) contacting said template with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing said second specified base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing said selected base, under conditions effective to produce a nucleic acid product terminating with a trinucleotide sequence of said first and second specified bases and said selected base; and
- 15 h) detecting said nucleic acid product under conditions effective to identify a selected trinucleotide sequence in said nucleic acid sample.

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25 72. The method of claim 15, further defined as a method for identifying a selected trinucleotide sequence of a first, second and third base in a nucleic acid template, said method comprising:

- 30 a) blocking said template by contacting with a first blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;

- (b) removing said first blocking composition from contact with said template;
- 5 (c) extending said template by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;
- 10 (d) removing said first extending composition from contact with said template;
- 15 (e) blocking said template by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said second base;
- 20 (f) removing said second blocking composition from contact with said template;
- g) contacting said template with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing the complement of said second base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said third base, under conditions effective to produce a nucleic acid product terminating with a trinucleotide sequence complementary to said first, second and third bases; and
- h) detecting said nucleic acid product under conditions effective to identify said selected trinucleotide sequence in said nucleic acid sample.

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73. The method of claim 72, comprising:

- a) blocking said template by contacting with a first blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;
- b) removing said first blocking composition from contact with said template;
- c) extending said template by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;
- d) removing said first extending composition from contact with said template;
- e) blocking said template by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said second base;
- f) removing said second blocking composition from contact with said template;
- g) further extending said template by contacting with a second extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said second base;
- h) terminating the reaction by contacting said template with a terminating composition comprising a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said third base, under conditions effective to produce a nucleic acid product terminating with a trinucleotide sequence complementary to said first, second and third bases; and

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i) detecting said nucleic acid product under conditions effective to identify a selected trinucleotide sequence in said nucleic acid sample.

5 74. The method of claim 1, further defined as creating a population of nucleic acid products terminated at four selected bases, comprising contacting a population of substantially double-stranded templates comprising at least a first break on at least one strand with a terminating composition comprising four terminating bases corresponding to said four selected bases, under conditions effective to produce a population of nucleic acid products terminated at four selected
10 bases.

15 75. The method of claim 74, further defined as a method for sequencing a nucleic acid comprising detecting said population of nucleic acid products terminated at four selected bases under conditions effective to determine the sequence of at least a portion of said nucleic acid.

20 76. The method of claim 75, further comprising contacting said template with at least four extending nucleotides.

25 77. The method of claim 74, wherein each of said four terminating bases comprise a distinct fluorescent label.

30 78. The method of claim 1, wherein said template is a covalently closed circular template.

79. The method of claim 1, wherein said template is a linear template.

80. The method of claim 1, wherein said template is created by cleavage from a precursor nucleic acid molecule.

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81. The method of claim 1, wherein said template is created by amplifying the template from a precursor nucleic acid molecule.

10 82. The method of claim 81, wherein said template is created by a temperature cycling
amplification method.

83. The method of claim 82, wherein said template is created by PCR.

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84. The method of claim 83, comprising:

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- a) contacting said precursor molecule with at least a first and a second primer that amplify said template when used in conjunction with a polymerase chain reaction, wherein at least one of said first or second primers comprises at least a first uracil base; and
- b) conducting a polymerase chain reaction to create said template.

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85. The method of claim 81, wherein said template is created by an isothermal amplification method.

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86. A method for sequencing a nucleic acid molecule, comprising:

- a) creating a population of substantially double-stranded nucleic acid templates from said nucleic acid molecule, each of said templates comprising at least a first random break on at least one strand;
- b) contacting said templates with an effective polymerase and a terminating composition comprising four distinct labeled or tagged terminating nucleotides, under conditions effective to produce a population of terminated nucleic acid products;
- c) detecting said terminated nucleic acid products under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid molecule.

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87. The method of claim 86, wherein said templates are contacted with said terminating composition in four distinct reactions, each of said reactions comprising only one of said four distinct labeled or tagged terminating nucleotide.

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88. The method of claim 86, wherein said templates are contacted with said terminating composition in a single reaction, and wherein each of said four terminating nucleotides comprises a distinct, fluorescent label.

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89. A method for sequencing a nucleic acid molecule, comprising:

- a) creating at least a first substantially double-stranded nucleic acid template from said nucleic acid molecule, the template comprising at least a first random break on at least one strand;
- b) contacting said template with an effective polymerase and at least a first extending and terminating composition comprising four extending deoxynucleotide triphosphates and a labeled or tagged terminating dideoxynucleotide triphosphate, under conditions effective to produce a population of terminated nucleic acid products;
- c) detecting said terminated nucleic acid products under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid molecule.

90. A method of sequencing a nucleic acid molecule by identifying at least a selected dinucleotide sequence comprising at least a first base and a second base, the method comprising:

- a) creating a population of substantially double-stranded nucleic acid template from said nucleic acid molecule, the templates each comprising a selected dinucleotide sequence on a template strand and comprising at least a first, random break on a non-template strand;
- b) blocking said templates by contacting with a blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;
- c) removing said blocking composition from contact with said templates;

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 - d) contacting said templates with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said second base, under conditions effective to produce a population of nucleic acid products in which the non-template strands terminate with a dinucleotide sequence complementary to said first and second base;
 - 10 e) detecting said nucleic acid products under conditions effective to identify said selected dinucleotide sequence in said nucleic acid templates; and
 - f) compiling the identified dinucleotide sequences to determine the contiguous nucleic acid sequence of at least a portion of said nucleic acid molecule.

91. The method of claim 90, further defined as a method for sequencing a nucleic acid molecule comprising identifying at least a selected trinucleotide sequence comprising at least a first, second and third base, wherein step (d) of said method comprises:

- 25 i) extending said templates by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;

ii) removing said first extending composition from contact with said templates;

iii) blocking said templates by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said second base;

- iv) removing said first blocking composition from contact with said templates;
- v) contacting said templates with at least a first extending and terminating composition comprising an extending deoxynucleotide triphosphate containing the complement of said second base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said third base, under conditions effective to produce a population of nucleic acid products in which the non-template strands terminate with a trinucleotide sequence complementary to said first, second and third bases;

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and wherein the selected trinucleotide sequences of the template strand are identified and compiled to generate at least a contiguous portion of the sequence of said nucleic acid molecule.

15 92. A method of mapping a nucleic acid; comprising:

- a) creating a population of substantially double-stranded nucleic acid templates from said nucleic acid comprising at least a first, random break on only one strand;
- b) contacting said population with an effective polymerase and at least a first degradable extension-producing composition comprising three non-degradable extending deoxynucleotides and one degradable nucleotide, under conditions and for a time effective to produce a population of degradable nucleic acid products comprising said degradable nucleotide;
- c) removing said degradable extension-producing composition from contact with said templates;
- d) contacting said population of degradable nucleic acid products with an effective polymerase and at least a first nondegradable extending and terminating

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composition comprising four non-degradable extending deoxynucleotides, at least one of said non-degradable extending deoxynucleotides comprising a detectable label or an isolation tag, under conditions and for a time effective to produce a population of terminated nucleic acid products comprising a degradable region and a nondegradable region;

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- e) contacting said population of terminated nucleic acid products with an effective amount of a degrading composition to degrade said degradable region, thereby producing nested nucleic acid products; and
 - 10 f) detecting said nested nucleic acid products under conditions effective to determine the position of said nucleic acid relative to said nucleic acid product.

15 93. A method of sequencing a nucleic acid molecule by identifying a selected dinucleotide sequence comprising a first base and a second base, the method comprising:

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- a) creating a substantially double-stranded nucleic acid template comprising at least a first random break on at least one strand, a selected dinucleotide sequence on a template strand and comprising an exonuclease-resistant nucleotide in the non-template strand, wherein the base of said exonuclease-resistant nucleotide is complementary to said first base;
 - 25 b) contacting said template with an amount of an exonuclease effective to degrade the non-template strand until the position of the exonuclease-resistant nucleotide;
 - c) removing said exonuclease from contact with said template;
 - 30 d) contacting said template with at least a first terminating composition comprising a tagged or labeled terminating dideoxynucleotide triphosphate containing the

complement of said second base, under conditions effective to produce a nucleic acid product terminating with a dinucleotide sequence complementary to said first and second base; and

- 5 e) detecting said nucleic acid product under conditions effective to identify said selected dinucleotide sequence in the template strand of said nucleic acid template.

10 94. A method of sequencing through a telomeric repeat region into a subtelomeric region, comprising:

- 15 a) providing a substantially double-stranded nucleic acid that comprises, in order, a terminal single-stranded telomeric overhang, a double-stranded telomeric repeat region and a double-stranded subtelomeric region;
- 20 b) contacting said nucleic acid with a composition comprising a primer that hybridizes to said single-stranded telomeric overhang, an effective polymerase, four extending nucleotides and at least a first tagged or labeled terminating nucleotide under conditions effective to produce a nucleic acid product extended from said primer into said subtelomeric region; and
- 25 c) detecting said nucleic acid product under conditions effective to determine the nucleic acid sequence of said telomeric overhang, said telomeric repeat region and at least a portion of said subtelomeric region.

30 95. A method of determining the length of a single-stranded overhang of a telomere, comprising contacting a telomere comprising a single-stranded overhang with an excess of a primer that hybridizes to said single-stranded overhang under conditions effective to allow

hybridization of substantially complementary nucleic acids, and quantitating the primers thus hybridized to said single-stranded overhang.

5 96. The method of claim 95, further comprising contacting the primers hybridized to said single-stranded overhang with a ligation composition in an amount and for a time effective to ligate said primers, and wherein the length of the ligated primers is quantitated.

10 97. A method of selecting a nucleic acid product terminated at a selected base, comprising creating a substantially double stranded nucleic acid template comprising at least a first break on at least one strand, and contacting said template with:

- 15 a) an effective polymerase and a terminating composition comprising at least a first terminating nucleotide, wherein the base of said terminating nucleotide corresponding to said selected base, under conditions effective to produce a nucleic acid product terminated at a selected base; or
- 20 b) an effective polymerase and an extending composition under conditions effective to produce a fully extended product only from a template that terminates at said selected base.

25 98. The method of claim 97, comprising creating a substantially double stranded nucleic acid template comprising at least a first random double stranded break.

99. The method of claim 98, further defined as a method for determining the position of a selected dinucleotide sequence of a first and second base in a nucleic acid template, said method comprising:

- 5 a) ligating a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand comprising a blocked 5' end and a 3' end comprising a hydroxyl group;
- 10 b) blocking said template by contacting with a first blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;
- 15 c) removing said first blocking composition from contact with said template;
- 20 d) extending said template by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;
- 25 e) removing said first extending composition from contact with said template;
- 30 f) blocking said template by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said second base;
- g) removing said second blocking composition from contact with said template;
- h) contacting said template with at least a second extending composition comprising four extending deoxynucleotide triphosphates, at least one of said extending deoxynucleotide triphosphates containing a tagged or labeled base, under

conditions effective to produce a fully extended tagged or labeled nucleic acid product with a dinucleotide sequence complementary to said first and second bases; and

- 5 i) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

100. The method of claim 98, further defined as a method for determining the position of a
10 selected trinucleotide sequence of a first, second and third base in a nucleic acid template, said
method comprising:

- 15 a) ligating a double-stranded nucleic acid segment to said double-stranded break,
said double-stranded nucleic acid segment comprising an upper strand comprising
a 5' end comprising a phosphate group and a blocked 3' end and a lower strand
comprising a blocked 5' end and a 3' end comprising a hydroxyl group;
- 20 b) blocking said template by contacting with a first blocking composition comprising
three dideoxynucleotide triphosphates that do not contain the complement of said
first base;
- 25 c) removing said first blocking composition from contact with said template;
- d) extending said template by contacting with a first extending composition
comprising an extending deoxynucleotide triphosphate containing the
complement of said first base;
- e) removing said first extending composition from contact with said template;

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- f) blocking said template by contacting with a second blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said second base;
 - 5 g) removing said second blocking composition from contact with said template;
 - h) extending said template by contacting with a second extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said second base;
 - i) removing said second extending composition from contact with said template;
 - j) blocking said template by contacting with a third blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said third base;
 - k) removing said third blocking composition from contact with said template;
 - l) contacting said template with at least a third extending composition comprising four extending deoxynucleotide triphosphates, at least one of said extending deoxynucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a trinucleotide sequence complementary to said first, second and third bases; and
 - m) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

101. The method of claim 98, further defined as a method of determining the position of a selected dinucleotide sequence comprising a first base and a second base in a nucleic acid template, the method comprising:

- 5 a) attaching a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand comprising a blocked 5' end and a blocked 3' end;
- 10 b) heating said template at a temperature effective to disassociate said lower strand of said adaptor;
- 15 c) annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base and a second additional 3' base complementary to said second base;
- 20 d) contacting said template with an extending composition comprising four extending deoxynucleotide triphosphates, at least one of said extending deoxynucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a dinucleotide sequence complementary to said first and second bases; and
- 25 e) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

102. The method of claim 98, further defined as a method of determining the position of a selected trinucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

- 5 a) attaching a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand comprising a blocked 5' end and a blocked 3' end;
- 10 b) heating said template at a temperature effective to disassociate said lower strand of said adaptor;
- 15 c) annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base, a second additional 3' base complementary to said second base and a third additional 3' base complementary to said third base;
- 20 d) contacting said template with an extending composition comprising four extending deoxynucleotide triphosphates, at least one of said extending deoxynucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a trinucleotide sequence complementary to said first, second and third bases; and
- 25 e) detecting said nucleic acid product under conditions effective to determine the position of said selected trinucleotide sequence in said nucleic acid sample.

103. The method of claim 98, further defined as a method of determining the position of a selected dinucleotide sequence comprising a first base and a second base in a nucleic acid template, the method comprising:

- 5 a) ligating a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand comprising a blocked 5' end and a blocked 3' end;
- 10 b) heating the ligated double-stranded nucleic acid segment at a temperature effective to disassociate said lower strand of said adaptor;
- 15 c) annealing a first single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said first oligonucleotide comprising the same nucleotide sequence as said lower strand;
- 20 d) blocking said templates by contacting with a first blocking composition comprising a dideoxynucleotide triphosphate that contains the complement of said first base;
- 25 e) removing said first blocking composition from contact with said templates;
- f) contacting said templates with at least a first extending composition comprising four deoxynucleotide triphosphates, one of said deoxynucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;
- 30 g) heating the templates at a temperature effective to disassociate said first single stranded oligonucleotide;

- 5
- h) annealing a second single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide sequence as said first single-stranded oligonucleotide plus a first additional 3' base complementary to said first base;
- 10
- i) blocking said templates by contacting with a second blocking composition comprising a dideoxynucleotide triphosphate that contains the complement of said second base;
 - j) removing said second blocking composition from contact with said templates;
- 15
- k) contacting said templates with said at least a first extending composition comprising four deoxynucleotide triphosphates, one of said deoxynucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;
- 20
- l) heating the templates at a temperature effective to disassociate said second single stranded oligonucleotide;
 - m) annealing a third single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide sequence as said second single-stranded oligonucleotide plus a second additional 3' base complementary to said second base;
- 25
- n) contacting said templates with at least a second extending and labeling composition comprising four deoxynucleotide triphosphates, at least one of which comprises a detectable label, under conditions effective to completely extend the non-template strand;

- o) contacting said templates with at least a first degrading composition under conditions effective to degrade the non-template strands containing a uracil base; and
- 5 p) detecting said nucleic acid products under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid templates.

104. The method of claim 98, further defined as a method of determining the position of a
10 selected trinucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

- 15 a) ligating a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand comprising a blocked 5' end and a blocked 3' end;
- 20 b) heating the ligated double-stranded nucleic acid segment at a temperature effective to disassociate said lower strand of said adaptor;
- c) annealing a first single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said first oligonucleotide comprising the same nucleotide sequence as said lower strand;
- 25 d) blocking said templates by contacting with a first blocking composition comprising a dideoxynucleotide triphosphate that contains the complement of said first base;
- e) removing said first blocking composition from contact with said templates;

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- 5 f) contacting said templates with at least a first extending composition comprising four deoxynucleotide triphosphates, one of said deoxynucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;

10 g) heating the templates at a temperature effective to disassociate said first single stranded oligonucleotide;

15 h) annealing a second single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide sequence as said first single-stranded oligonucleotide plus a first additional 3' base complementary to said first base;

20 i) blocking said templates by contacting with a second blocking composition comprising a dideoxynucleotide triphosphate that contains the complement of said second base;

25 j) removing said second blocking composition from contact with said templates;

k) contacting said templates with said at least a first extending composition comprising four deoxynucleotide triphosphates, one of said deoxynucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;

l) heating the templates at a temperature effective to disassociate said second single stranded oligonucleotide;

m) annealing a third single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide

sequence as said second single-stranded oligonucleotide plus a second additional 3' base complementary to said second base;

- n) contacting said templates with said at least a second extending composition comprising four deoxynucleotide triphosphates, one of said deoxynucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;
- o) heating the templates at a temperature effective to disassociate said third single stranded oligonucleotide;
- p) annealing a fourth single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide sequence as said third single-stranded oligonucleotide plus a third additional 3' base complementary to said third base;
- q) contacting said templates with at least a third extending and labeling composition comprising four deoxynucleotide triphosphates, at least one of which comprises a detectable label, under conditions effective to completely extend the non-template strand;
- r) contacting said templates with at least a first degrading composition under conditions effective to degrade the non-template strands containing a uracil base; and
- s) detecting said nucleic acid products under conditions effective to determine the position of said selected trinucleotide sequence in said nucleic acid templates.